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Varying levels of clonality and ploidy create barriers to gene flow and challenges for conservation of an Australian arid-zone ecosystem engineer, Acacia loderi

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Abstract

Acacia loderi, the ecosystem engineer of the endangered Acacia loderi Shrublands in arid eastern Australia, spans a persistent (> 15 000 year) but poorly studied landscape feature, the Darling River. We investigated the genetic structure of 19 stands of eight to > 1000 plants separated by < 300 km to test for variation in life histories between semi-arid and arid stands to the east and west of the Darling River, respectively. Eight of nine stands east of the Darling were exclusively sexual, whereas most of those to the west were clonal. Three western stands were monoclonal, two were polyploid, and one was a diverse mix of diploid and triploid phenotypes. Bayesian analysis revealed a complex genetic structure within the western stands, whereas the eastern stands formed only two genetic clusters. Conservation of small stands may require augmentation of genotypic diversity. However, most genotypic diversity resides within the eastern stands. Although arid zone stands of A. loderi are not always clonal, clonality and polyploidy are more common in the arid west. Clear demarcation of life histories either side of the Darling River may reflect ancient or contemporary effects of physical disturbance associated with the river channel, or cryptic environmental differences, with sexual and asexual reproduction, respectively, at a selective premium in the semi-arid east and arid west. The restricted distribution of clones and variation in clonality and polyploidy suggests that smaller stands may be vulnerable and warrant individual management.

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Varying levels of clonality and ploidy create barriers to gene flow and challenges for conservation of an Australian arid-zone ecosystem engineer, *Acacia loderi*

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ABSTRACT

Acacia loderi, the ecosystem engineer of the Australian Endangered arid zone *Acacia loderi* Shrubland, spans a persistent (>15000 yr.) but poorly studied landscape feature, the Darling River. We investigated the genetic structure of 19 stands of 8 to > 1000 plants separated by < 300 km to test for variation in life-histories between semi-arid and arid stands to the east and west of the Darling River, respectively. Eight of nine stands east of the Darling were exclusively sexual whereas most of those to the west were clonal. Three western stands were monoclonal, two were polyploid, and one was a diverse mix of diploid and triploid phenotypes. Bayesian analysis revealed a complex genetic structure within the western stands whereas the eastern stands formed only two genetic clusters. Conservation of small stands may require augmentation of genotypic diversity. However, most genotypic diversity resides within the eastern stands. Although arid zone stands of *A. loderi* are not always clonal, clonality and polyploidy are more common in the arid west. Clear demarcation of life-histories either side of the Darling River may reflect ancient or contemporary effects of physical disturbance associated with the River channel, or cryptic environmental differences, with respectively sexual and asexual reproduction at a selective premium in the semi-arid east and arid west. The restricted distribution of clones and variation in clonality and polyploidy suggests that smaller stands may be vulnerable and warrant individual management.

Keywords: *Acacia loderi*; Darling River gene flow; genetic diversity; habitat fragmentation; perennial plant; polyploidy; sexual and asexual reproduction

INTRODUCTION

The capacity of populations to evolve and persist in the face of natural disturbance or anthropogenic habitat degradation is strongly determined by their levels of genotypic diversity (Barrett & Kohn, 1991; Ellstrand & Elam, 1993; Frankham, 2005). However, the genotypic composition of populations of many plant species inevitably reflects the interactions of a range of ecological and evolutionary processes. Arguably, for plants, the most important determinant of genotypic diversity is their relative investment in sexual and asexual reproduction (Williams, 1975; Maynard Smith, 1978), and the spatial extent over which pollen or seed are dispersed (Loveless & Hamrick 1984; Ghazoul, 2005). Intriguingly, reviews by Kearney (2003, 2005) suggest that in the central and western Australian arid-zone the reproduction of many plants and animals occurs by parthenogenesis, with many polyploid species appearing to have lost the capacity to reproduce sexually (see also Kearney & Blacket, 2008; Clarke *et al*., 2012). Such taxa may therefore be less resilient to disturbance than obligately sexual congeners that occur in more mesic or temperate areas in Australia.

The link between resilience and reproductive mode and contemporary and historical processes are poorly understood for most of Australia's arid and semi-arid plant species. Many species occupy ancient landscapes (see Gale, 1992; Pillans, 2007) within which spatially separated stands may previously have had strong genetic connections over vast geographical areas (e.g. Byrne, 1999). However, neither variation in life histories or levels of connectedness among stands has been assessed for the majority of species. Currently, some species form vast and thriving stands, while anthropogenic impacts such as land clearing for agriculture and overgrazing by stock and feral herbivores have confined others to small and potentially isolated remnant fragments. Grazing and the increased isolation of stands have the potential to influence genotypic diversity directly through decreased population size, and indirectly through reduction in inter-population gene flow or alteration of reproductive tactics. This influence will be greatest if, as demonstrated in other terrestrial systems, grazing promotes asexual reproduction (albeit through vegetative spread rather than apomictic parthenogenesis) (e.g. Evju *et al*., 2011) and/or disrupts plant-pollinator interactions (Yoshihara *et al.*, 2008; Kimoto *et al.*, 2012). Nevertheless, it is also possible that prior to European settlement in Australia, many arid-zone species already exhibited low levels of connectedness, reflecting the spatially patchy distribution of suitable habitat (Hopper, 1979;

Coates, 2000; Byrne & Hopper, 2008; O'Brien, Denham & Ayre, 2014) and the presence of poorly understood landscape features that may act as barriers to dispersal or demarcate strongly contrasting but cryptic environmental conditions, such as those created by Australia's complex system of inland rivers.

In contrast to the importance that has been placed on landscape features, principally rivers and mountain ranges, in shaping the distribution and evolution of species' worldwide (see Soltis *et al*., 2006; Shafer *et al*., 2010; Turchetto-Zolet *et al*., 2013 for recent reviews) there is almost no recognition of their presence within Australia's vast arid interior (but see Byrne *et al*., 2008). Australia's largest inland river system, the Murray-Darling drains much of inland southeastern Australia, and for millions of years, the principal rivers of this vast system have dissected this inland region known as the Murray Darling Basin (MDB). The Darling River (DR), flowing > 1000 km from southern Queensland to southwest Victoria has a persistent river channel and associated flood plain that, for > 15,000 years has followed its present course and separated arid western and semi-arid eastern terrestrial biota of the MDB. Located in the south and southeast of the MDB, the Lachlan River and its distributary channel, Willandra Creek, further fragments terrestrial habitats in this region (see Williams *et al*., 1991; Bowler, Kotsonis, & Lawrence, 2006) (refer to Fig. 1 a & b below). While Smissen *et al*., (2013) revealed the Murray-Darling as a dispersal conduit for terrestrial vertebrates, to our knowledge, there are no studies that have examined the role of the DR in potentially separating the biota of the eastern semi-arid from its drier arid interior.

Acacias are common within Australian arid- and semi-arid zone plant communities (Beadle, 1981), and as ecosystem engineers provide habitat, food resources and modify the harsh environment by providing shade, increasing soil nutrient levels, and improving rainfall infiltration (Tongway & Ludwig, 1990; Facelli & Brock, 2000; Dunkerly, 2002; Prider & Facelli, 2004). However, many *Acacia* species are now considered vulnerable or threatened because of apparently infrequent sexual reproduction and chronic recruitment failure that may result from low genetic and genotypic diversity together with the impacts of overgrazing. Reliance on clonal reproduction may reflect the proximate loss of sexual reproduction caused by anthropogenic ecosystem degradation. Alternatively, it may be a landscape-scale condition that has evolved in response to Australia's long history of aridification (Bowler, 1976), or be at a selective premium in some locations, and hence the degree of clonality may

vary throughout a species' range. Indeed, sexual recruitment may be rare because clonal reproduction provides sufficient recruits to sustain populations and minimises local extinction. Some genotypes may be able to respond to rare boom conditions by mast flowering and produce subsequently large seed crops to maximise rare recruitment opportunities, while others may be constrained to low levels of sexual reproduction (Eckert 2001).

Within the southeastern part of the Australian arid zone, few authors have investigated whether plant species in general, and *Acacia* in particular, exhibit obligate asexuality or polyploidy or both. In this area, long-term demographic studies indicate that some species recruit exclusively from seed (assumed to be sexually generated) whereas others may be almost entirely reliant on asexual reproduction via the production of root suckers (e.g. Auld, 1993, 1995). However, without genetic confirmation of reproductive modes, the potentially great longevity of these plants (Auld & Denham, 2001), the repeated cycles of 'boom and bust' flood and drought events that characterise the Australian arid zone (Greenville, Wardle, & Dickman, 2012; Wardle, Pavey, & Dickman, 2013), and current intense grazing pressure from both native, feral, and domestic herbivores (Auld, 1990; Tiver & Andrew, 1997; Denham & Auld, 2004) confound attempts to predict the importance of each reproductive mode in determining the genetic makeup of populations. Indeed, there are species such as *A. ligulata* A. cunn. ex Benth. and *A. victoriae* Benth., which are highly fecund, form large dense stands, and display frequent sexual recruitment (Grice, Westoby, & Torpy, 1994; Auld, 1995; Denham & Auld, 2004). In contrast, nearly all populations of the broadly sympatric *A. carneorum* Maiden have not set seed for 20 years, with all recruitment involving asexual reproduction by means of root suckering (Auld, 1993; Denham & Auld, 2004).

Acacia loderi Maiden is the dominant canopy tree within the *Acacia loderi* Shrubland Endangered Ecological Community (New South Wales Government, 2011) and the available evidence suggests populations display great variation in their reliance on asexual reproduction through suckering, although most populations are considered in decline (Auld, 1995; New South Wales Government, 2011). Suckering may contribute to the resilience and persistence of *A. loderi* within the contemporary landscape, because they can be produced almost continuously, and while subject to grazing, can re-sprout and hence re-establish year-after-year (A. Denham unpublished data). In contrast, seedlings (sexual recruits) are produced only

rarely (Auld, 1995a), possibly in years with favourably high rainfall (A. Denham pers. obs.), and are highly susceptible to mortality due to desiccation and herbivory (Auld & Denham, 2001). Seedlings are highly palatable and are likely eaten by livestock (sheep and cattle) and exotic feral herbivores (rabbits and goats), as well as native herbivores such as kangaroos (Auld, 1995b). The widespread, but patchy contemporary distribution of *A. loderi* in the semi-arid region east of the DR, and in the arid region west of the DR (Beadle, 1981; Pickard & Norris, 1994), implies that it has experienced a complex history of possible range expansion and contraction and shifting patterns of fragmentation that reflect both a series of marine inundations and shifts in the importance and location of river channels in response to changing climate through the Plio-Pleistocene (Bowler, Kotsonis, & Lawrence, 2006). Throughout its range, and within our study area in the southwestern MDB, *A. loderi* occurs on ancient solonized brown and duplex soils and calcareous red earths in relict drainage depressions, sandy rises, and plains, but is absent from the grey cracking clays that have been transported from the far north of the MDB by the river system, and which are associated with riverine floodplains and lakes (Beadle, 1981; Pickard & Norris, 1994).

In this study, we examined the distribution of genetic variation throughout the range of *A. loderi*, compared the genetic diversity within individual stands, and estimated the relative importance of sexual and asexual reproduction. We used these data to explicitly determine whether the connectedness, spatial genetic structure, and life-histories exhibited by *A. loderi* stands vary between the arid eastern and semi-arid western regions demarcated by river channel and extensive floodplains of the DR.

MATERIALS AND METHODS

Basic biology and stand structure of *Acacia loderi*

Acacia loderi stands typically comprise up to 100's of 3 to > 8 m high trees, each separated by several tens to hundreds of meters. In some stands, however, an abundance of smaller (< 1 m high) putative root suckers surround remnant trees, while very rarely, stands comprise dense clusters of 1 to 2 m high trees with overlapping canopies.

Virtually nothing is known about the pollination biology or seed dispersal mechanisms of *A. loderi*. Showy inflorescences arranged on branchlets together with synchronous flowering imply that generalist insect visitors (e.g. native and exotic bees, and native flies or

wasps) are probably important pollinators (Bernhardt, 1987; Gilpin *et al*., 2014). As for other acacias worldwide, seeds possess a small nutritious aril, suggesting that either ant or small avian vectors may be involved in seed dispersal (Berg, 1975; Davidson & Morton, 1984; Auld, 1995a). In addition, the seeds are contained in seedpods, which are held on the maternal plant until they are mature, at which point the pods dehisce, revealing the seed/s, before both the pod (often with attached seed/s) falls to the ground forming a dense litter (as per *A. eriopoda* Maiden & Blakely - Davidson & Morton, 1984). Seed may occasionally be dispersed by larger seed feeders such as emus (e.g. He *et al*., 2009; Calviño-Cancela, 2011), while whole pods may be dispersed by wind or water.

Population sampling

We sampled nineteen stands of *A. loderi* separated by up to 300 km within the core of its known range in the vicinity of Kinchega National Park (Kinchega NP) (Fig. 1b; Table 1). There were 9 stands located in the semi-arid east of the DR, and ten in the arid west. Within each stand we haphazardly sampled phyllodes from between 8 and 62 stems (total n = 579). We deliberately targeted large stems, separated by > 10 m (and in some cases > 100 m) to reduce the probability of sampling the ramets of genets. Our sampling therefore aimed to capture as much as possible the available genotypic diversity contained in each stand. Sample sizes varied with the absolute number of trees in each stand; sample sizes < 30 comprised all available stems other than those forming tight clusters of apparent vegetative suckers around mature adult plants (refer above, 'basic biology and stand structure'. We generated eight-locus microsatellite data for all sampled stems. DNA extraction and genotyping followed standard laboratory protocols, and the primer set comprising 8 microsatellite loci is described in Roberts *et al*., (2013).

Identification of clones and levels of genetic diversity within stands

Because *A. loderi* can reproduce both sexually and asexually (by means of vegetative root suckers) we used GenClone (Arnaud-Haond & Belkhir, 2007) to estimate the unique genotype probability (P_{gen}) and hence G, the number of distinct multilocus genotypes (MLG) or genets contained in each stand. We then used these data to estimate clonal richness, R, and clonal heterogeneity (calculated as the complement of Simpson's index, D^*) within stands (Dorken & Eckert, 2001). Clonal richness (R) represents the ratio of the observed number of

MLGs (minus one) to stems (minus one), and ranges between zero and one, with values of zero indicating that all samples are identical, and one indicating every stem is unique. Because *R* is essentially a measure of the proportion of the sample that is variable, it provides no information about the rate of recurrence of specific clones within a sample; that is, for a given estimate of *R*, a sample may include either a small number of clones found at high frequency with others that are rare, or all clones could be equally represented. We therefore used the unbiased estimator of the complement of Simpson's index (D^*) as a measure of clonal heterogeneity within stands. This index describes the probability that two randomly chosen stems represent different MLGs (Arnaud-Haond *et al*., 2007).

Before generating estimates of clonal richness, *R*, and clonal heterogeneity (calculated as the complement of Simpson's index, D^*) within stands (Dorken & Eckert, 2001), we assessed whether putatively distinct MLGs were actually unique. This was considered necessary because somatic mutation/s or genotyping errors (e.g. associated with mis-scoring electropherograms or null alleles) can produce slightly genetically dissimilar pairs of MLGs, inflating numbers of MLGs estimated per stand and hence estimates of both *R* and D^*. We examined a frequency distribution of microsatellite genetic distance among all genotyped stems (Arnaud-Haond *et al*., 2007). This distribution was essentially unimodal, although there were a small number of outliers distributed at the lower end of the range of genetic distances, indicating very slight genetic dissimilarity among a small number of genotyped stems (data not presented). We examined the genotypes of each MLG pair exhibiting very low genetic dissimilarity. Most were contained in clonal western stands, and in the majority of cases, slight genetic dissimilarity was attributable to either a decrease or increase in one dinucleotide microsatellite repeat motif at a single locus. This implies that these slightly distinct MLG are most likely produced by asexual reproduction, but one or the other of the pair have a somatic mutation, resulting in a slightly distinct copy of essentially the same multilocus lineage (MLL) (Arnaud-Haond *et al*., 2007). Importantly, this can be evaluated by excluding the locus exhibiting the slightly different genotype and calculating the likelihood that the *n* (where $n = 1, 2, 3 \ldots i$) copies of the MLG are the product of independent episodes of sexual reproduction, P_{sex}. Where $P_{sex} < 0.001$ it is highly improbable that *n* repeated MLG are the product of sexual reproduction alone, indicating a contribution of asexual reproduction to genotypic diversity. For all cases, P_{sex} values calculated after excluding the slightly different locus were highly significant (< 0.001), implying that slightly dissimilar MLG pairs are

essentially identical, and we have treated them as such for the purpose of estimating overall clonal richness and the level of clonal heterogeneity within each stand (R & D^*) (Arnaud-Haond *et al*., 2007).

Accurately identifying MLGs requires sufficiently variable and hence powerful molecular markers. We tested the efficiency of our eight microsatellites to distinguish MLGs contained in each stand using a permutation procedure (based on 1000 replicates) that resampled combinations of 1 – l, where l is the number of loci available, allowing estimates of the average ± SE MLGs detected for increasing numbers of loci. To compare among populations, we calculated and plotted estimates of R, as a function of the number of loci used to distinguish MLGs. For all stands, these plots revealed a curve approaching an asymptote, implying that variation is sufficient to resolve all distinct MLG (data not shown).

Levels of genetic diversity within stands

For each stand, we calculated the average number of alleles per locus (A) allelic richness (A_R) standardised to n = 4 (the smallest sample size after the exclusion of all clonal replicates), and observed and expected heterozygosity (H_o & H_e). We also tested for single-locus departures from Hardy-Weinberg equilibria and calculated the average inbreeding coefficient, F_{IS}, for each stand. This was done to infer the mating system that produced the adult plants. Consistent deficits or excesses of heterozygosity reflected by respectively positive and negative values of F_{IS} are consistent with inbreeding or outbreeding whereas a mixture of heterozygous excesses and deficits together with the presence of replicated MLG is consistent with clonal reproduction.

Polyploid phenotypes west of the Darling River

In diploids, microsatellite genotypes are typically visualised as one or two discrete profiles or peaks on electropherograms representing respectively homozygotes and heterozygotes. However, polyploid genetic systems are characterised by the presence of complex electropherograms, reflecting the presence of more than two alleles per locus. Our genetic surveys revealed two stands comprising putative polyploid plants. Both were located west of the DR (stands 12 & 18, Table 1). Polyploidy presents several challenges to data scoring, analysis, and interpretation, because of uncertain origins of the increased number of chromosomes (i.e. auto- cf. allopolyploidy). We therefore excluded these two stands from

many of our analyses, although we describe patterns of 'phenotypic diversity' within these stands (these are not genotypes because we could only record the presence and identity of alleles at each locus, not the number of copies of each allele).

Population differentiation and spatial genetic structure

We assessed genetic population structure of *A. loderi* using STRUCTURE (Ver. 2.3.4, Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, & Pritchard 2003, 2007) and TESS (Chen *et al*., 2007; Durand, Gaggiotti, & François, 2009). While both programs employ individual-genotype-based Bayesian clustering algorithms, it is not possible to incorporate geographic coordinates into analyses in STRUCTURE. In contrast, within TESS it is possible to analyse genotype data in combination with each individual's geographical coordinates. The inclusion of geographical coordinates in analyses demonstrably improves the inference of genetic population structure (Chen *et al*., 2007; François & Durand, 2010).

For the analysis in STRUCTURE (Ver. 2.3.4, Pritchard, Stephens, & Donnelly 2000; Falush, Stephens, & Pritchard 2003, 2007), we used the admixture model to calculate individual q_i-values, as well as the log probability or likelihood of K genetic clusters within the genotype data set, using values of K between 1 and 30. We used the correlated allele frequency and admixture model and default program parameters for all other settings. Data were collected for 10^4 iterations in a burn-in, followed by a run of 10^5 iterations. There were 20 replicate runs for each value of K. The ΔK statistic of Evanno, Regnaut, & Goudet (2005) was used as a formal estimator of the most likely number of genetic clusters detectable by STRUCTURE, as implemented in the program STRUCTURE HARVESTOR (available at http://taylor0.biology.ucla.edu/structureHarvester/ Earl & von Holdt, (2012). The admixture estimates for each individual (q_i-values) for the 20 runs for the selected K were averaged using the program CLUMPP (v 1.1.2) (Jakobsson & Rosenberg, 2007). We used the 'greedy' algorithm, based on 10^5 random input orders. The results were visualised using DISTRUCT (v 1.1) (Rosenberg 2004). For the analysis in TESS (Chen *et al*., 2007; Durand, Gaggiotti, & François 2009), we used the conditional autoregressive model (CAR) admixture model, setting the spatial interaction parameter or weighting of the geographic coordinates to 0.6. We performed 100 replicates of 10^4 sweeps each, disregarding the first 30 000 sweeps as burn-in, for values of K between 1 and 30. The most likely number of genetic clusters was inferred based on the Deviance Information Criterion (DIC) as recommended by the authors.

Estimates of mean DIC (based on 100 replicate runs) were plotted against K, and the K at which the DIC first reached an asymptote was considered to be the number of detectable genetic clusters within the data. We similarly used CLUMPP and DISTRUCT to calculate and visualise the average estimate of ancestry, in this instance, the 20 runs with the lowest DIC for the selected K were averaged and plotted.

We used Weir and Cockerham's (1984) formulation of standardised genetic variance, F_{ST}, and the program FSTAT (Goudet, 1995) to determine how genetic variation was partitioned among sexually derived stands located to the east of the DR (refer to Results below). We also calculated values of pairwise F_{ST} using FSTAT, and then tested for isolation by distance (using the IBD Web Service, http://ibdws.sdsu.edu/ Jensen, Bohunk, & Kelley, 2005) based on a Mantel test for matrix correlation between the matrices of $F_{ST}/(1-F_{ST})$ and geographic distance among stands.

RESULTS

Life-history variation within and among stands

Distinct differences in life-history were evident within and between the two major groups of stands sampled to the east and west of the DR. Western stands displayed strikingly greater clonality than eastern stands. In addition, two of the western stands, and none of the eastern stands, displayed at least some polyploid phenotypes (Table 1).

Eight of nine stands sampled to the east of the DR were apparently exclusively diploid and showed virtually no evidence of asexual recruitment. Indeed, these stands displayed relatively high levels of genotypic diversity and genotype frequencies consistent with exclusively sexual recruitment and almost every stem (243 of 248) genotyped displayed a distinct MLG. Unsurprisingly, estimates of clonal richness, R, and, clonal heterogeneity, D^*, ranged between 0.93 and 1. All eight stands displayed almost identical values of F_{IS} consistent with expectations for a mixed mating system and moderate inbreeding. The overall mean $F_{IS} \pm$ SE (n = 8) was 0.331 ± 0.017, with values for individual stands ranging from 0.237 to 0.382 (Table 2).

In sharp contrast to the genotypic structure within the eastern stands all of the ten western stands were clonal, with two stands displaying evidence of both clonality and a

triploid genetic system. Three of eight apparently diploid western stands comprised single genets (R & D^* = 0: Kinchega National Park Far North-West; Kinchega National Park South-West; Wilcannia), and there were two other stands in which we only detected two genets (Kinchega National Park South Big Dune, R & D^* = 0.143 & 0.536, and Popiltah R & D^* = 0.063 & 0.118, respectively). The remaining three diploid western stands were moderately clonal, with R and D^* values ranging from 0.377 to 0.828 and 0.920 to 0.982, respectively. Overall, only 79 distinct genotypes were detected within the 257 (putative diploid) western individuals surveyed. For all stands in which ramets of genets were detected, P_{sex} estimates indicated that it was highly improbable that these copies were sexually derived. All P_{sex} values were highly significant (< 0.001) (Table 1). In addition, plants within East-West Road (Kinchega NP) and Middlecamp were putatively clonal polyploids that displayed up to three alleles per locus, for one locus (this differed between stands). Of the 30 stems genotyped in East-West Road, only a single putative multilocus phenotype was detected. In contrast, Middlecamp contained 14 distinct phenotypes (from n = 30 stems genotyped). This included 12 putative diploids and 2 clonal triploids (8 & 10 copies of each clone).

The genotypic composition of the remaining eastern stand within Mungo National Park, like the majority of western stands, was highly clonal and comprised a single genet (R & D^* = 0). The composition of this stand may possibly have been influenced by its isolation from the majority of eastern stands by yet another riverine barrier, the Willandra Creek/Lakes System of the Lower Lachlan River.

Genetic diversity and fine-scale population structure within stands

Both the fine-scale genetic structure and genetic diversity of *A. loderi* stands inevitably reflected their underlying modes of reproduction and recruitment.

Levels of A_R (corrected for the number of unique genotypes/stand) varied sharply among stands, ranging from means ± SE of 3.7 ± 0.3 and 4.5 ± 0.4 within the genotypically diverse sexually derived (principally eastern) stands, to 1.4 ± 0.2 and 2.0 ± 0.3 within clonal stands west of the DR. Mean expected H_e based on all genets (unique genotypes) within each stand was always greater within sexually than clonally derived stands, ranging respectively between 0.69 ± 0.04 and 0.78 ± 0.04 and 0.19 ± 0.09 and 0.58 ± 0.89 (Table 2).

Although our sampling was designed primarily to address landscape-scale hypotheses, it is pertinent that in all stands, genets formed relatively discrete clusters or islands of stems within the landscape and hence were not intermingled. Across all stands, we estimate that the greatest area occupied by an individual genet was 200,000 m^2 (the monoclonal stand, Far North-West, Kinchega NP). However, within multi-clonal stands (principally Omega, Kinchega NP), the area covered by distinct genets was generally much smaller, ranging between 19 and ~4000 m^2. All distinct genets were confined to a single stand.

Population differentiation and spatial genetic structure

Our Bayesian analyses of broad-scale patterns of allelic variation among the 17 diploid stands revealed a complex genetic population structure that partially reflects the separation of the eastern and western stands, and greater genetic differentiation among the more clonal western stands. In all analyses, the eastern sexually derived stands displayed relatively little genetic structure, implying that pollen or seed dispersal has maintained strong connections among these widely separated stands.

It is perhaps unsurprising, given the considerable life-history variation that neither our analysis with STRUCTURE without *a priori* knowledge of each plant's stand of origin, nor TESS incorporating the geographic coordinates of each individual, revealed a simple or consistent pattern of genetic differentiation. We initially analysed our data-set using STRUCTURE without the incorporation of spatial priors to test for the presence of two clusters representing eastern and western groupings of stands. However, while this revealed that 2 or 3 clusters were the best supported outcome, these did not simply separate eastern and western stands. The analysis using TESS (and STRUCTURE incorporating a spatial prior – data not displayed) best supported inferences of 9 or 10 clusters of genotypes (Fig. 2). Although inspection of Fig. 2 shows that most plants within the 8 eastern stands are strongly associated with one of two clusters (indicated by the pink and blue shading), the majority of stands contain individuals representing these clusters. Moreover, the two more arid stands i.e. stands 3 & 8 (Fig. 1b), which are found to the east of the DR but are nearer to the DR/ DR floodplain region and are represented in Fig. 2 by blue shading, are more genetically similar to each other than they are to the other six eastern stands. Genotypes representing this blue cluster are well represented within several of the otherwise more highly differentiated western stands (Fig. 2). Estimates of ΔK, which were used to distinguish the best supported number

of genetic clusters, for respectively two and three clusters, were 73 and 90, with all values of ΔK for between 4 and 30 clusters < 2.5. A plot of the mean DIC (based on the output from TESS) with respect to between 1 and 30 genetic clusters reached an asymptote at 9 – 10 clusters of plants, implying much more genetic subdivision than was identified by STRUCTURE. This level of discrepancy with respect to the numbers of well supported clusters is not uncommon when comparing Bayesian analyses with and without priors, but here highlights diverse evolutionary histories of these stands, reflecting possibly both the allopatric divergence of eastern and western stands and the inevitable founder effects associated with the formation or maintenance of western stands by small numbers of genets.

Analysis of the eight sexually derived stands east of the DR confirmed a slight but significant differentiation of allele frequencies within these stands (F_{ST} = 0.043 ± 0.010; P < 0.05). This relatively low differentiation was reasonably consistent across loci (F_{ST} range = 0.024 – 0.100). All pairwise estimates of F_{ST} among stands revealed similarly low but statistically significant differentiation (range: 0.007 – 0.084, 27/28 comparisons P < 0.05). However, we detected no isolation by distance within these eastern stands separated by ≥ 200 km (Z = 113, r^2 = 0.020, P = 0.286).

DISCUSSION

The genetic structure of *A. loderi* east of the Darling River demonstrates that widely distributed semi-arid *Acacia* can form vast, strongly interconnected stands. Indeed, while the vulnerable *A. loderi* Shrublands may now be subject to effects of anthropogenic fragmentation including increased isolation, we found that eight stands separated by up to 200 km were genetically diverse, and displayed the low levels of genetic subdivision typical of plants with mixed mating systems and effective seed- or pollen- mediated gene dispersal (Ghazoul, 2005). Interestingly, our findings lend support to Kearney's (2003 & 2005) observation that Australia's arid-zone supports an unusually high frequency of clonal and polyploid taxa. Kearney highlighted an increased incidence of asexual reproduction via parthenogenesis with increasing aridity. This has been reported for the widespread arid zone *Acacia aneura* F. Muell. ex Benth. species complex known as mulga (Miller, Andrew, & Maslin, 2002; Andrew *et al*., 2003), and clonality is common in other arid/semi-arid zone plants, such as Mallee eucalypts (Kennington & James, 1997; Rossetto *et al*., 1999) and *Senna* (Holman & Playford, 2000). Our survey of genetic and life-history variation revealed

that the Darling River demarcates a major disjunction in life-histories, for *A. loderi.* Indeed, we found that more arid western (i.e. west of the DR) stands of *A. loderi* were typically clonal, but we infer that asexual reproduction occurs by root suckering. This difference in the life-histories of e.g. mulga and *A. loderi* may reflect both the low frequency of suckering in mulga and the potentially complex polyploid history of these taxa. We also found that two of the more western stands of *A. loderi* were both clonal and polyploid, and it therefore is possible that mulga and *A. loderi* are displaying similar responses to past or changing conditions within the arid zone. Most strikingly, our study provides the first example of an inland river system demarcating sharply contrasting patterns of life-history variation, with the DR and its floodplain of unsuitable soils apparently separating sexually generated eastern and predominately asexually generated western stands of *A. loderi*.

The Darling River; a potential biogeographic barrier

The phylogeographic literature includes many striking effects of rivers as ancient and contemporary barriers to the dispersal of plants and animals (Soltis *et al*., 2006; Shafer *et al*., 2010; Turchetto-Zolet *et al*., 2013). However, many well supported biogeographic barriers include rivers with channels/floodplains that are relatively minor when compared with the DR. Curiously, the potentially major importance of rivers, including the DR, has been largely neglected in Australia, although Smissen *et al*., 2013 demonstrated their importance as conduits for dispersal in a lizard (*Varanus varius*). This neglect may reflect the arid nature of the Australian continent and the fact that inland rivers such as the DR vary enormously in flow rates, and indeed, may fail to flow in some years. Importantly, however, despite variation in flow rates, which may make barrier he DR highly permeable to more mobile taxa, for some terrestrial plants, the DR floodplain and associated grey clay soils may represent a persistent barrier to dispersal. Indeed, the width of the DR (refer to Fig. 1) typically varies from 10 to > 50 km (Kingsford *et al*., 2004) and with the addition of the Willandra system in the south of the MDB, has almost certainly caused fragmentation of *A. loderi*'s predominant red soil habitat. Surprisingly, not only has there been little focus on the importance of Australia's arid rivers as potential biogeographic barriers, but there are few other population genetic studies of plants within this region. Taxonomically broad studies are needed to fully estimate the importance of the DR and other rivers in arid Australia.

Geographic scale of variation in life-histories and genetic variation within stands

Our genetic data confirms that *A. loderi* employs varying contributions of sexual and asexual reproduction for recruitment. Indeed, this life-history variation was extreme, with some stands monoclonal, while others contained entirely unique genotypes. While the mechanism driving a switch from reliance on sexually generated recruits to suckering is unknown, it is apparent that this is not simply a response to anthropogenic disturbance through habitat fragmentation and increased herbivory. While all stands arguably have comparable histories of disturbance following the imposition of agricultural activity (Pickard, 1991), we found that most of the clonal stands (including the two clonal polyploid stands at East West Road, Kinchega NP, and Middlecamp) occurred to the west of the DR, or in the case of Lake Mungo, were within a fragmented riverine landscape formed by the Willandra Creek/Lakes System of the Lower Lachlan River. Superficially, this pattern of life-history variation provides support for Kearney's (2003, 2005) findings of a high incidence of clonality within the arid zone, for a range of taxa. However, it is possible that in the case of *A. loderi*, this reflects a history of physical disturbance associated with the river rather than a response to increasing aridity. While Kearney hypothesised that parthenogenic lineages might be more successful than sexual lineages within the arid-zone (implying that there is a benefit accruing from the clonal replication of embryos), for *A. loderi* it seems likely that the switch from sexual reproduction to vegetative suckering may reflect other selective pressures, such as size-dependent escape from herbivory or desiccation, or indeed, more direct effects of intermittent rainfall that could prevent seedling establishment. However, geological evidence suggests that the channel of the DR has followed the same approximate path for at least 15,000 years (Williams *et al*., 1991; Bowler *et al*., 2006) providing considerable time for the development of evolutionary adaptation of isolated stands.

In contrast to the largely clonal stands of the west, *A. loderi* stands to the east were the product of sexual recruitment and showed remarkably little variation in mating systems, inferred from departure from Hardy-Weinberg equilibria of adults. All stands displayed similar allelic diversity and inbreeding coefficients consistent with moderate inbreeding. While adult genotype frequencies are commonly used to infer the mating system that led to their recruitment, this approach assumes that mating systems have reached an equilibrium state (Hedrick, 2005) and we are conscious that although many of the sampled stems may pre-date the anthropogenic disturbance of the region, they may also reflect continuing effects of fragmentation or disruption of pollinator activity.

Population differentiation and spatial genetic structure

Examination of population differentiation across the 17 diploid stands revealed considerable life-history variation within and among eastern and western stands, including large variation in effective population sizes among the western stands, together with a complex history of isolation of groups of stands by the DR. Although the historic diversity of western stands is unknown, the nature of existing multi-clonal stands, in which each clone typically occupies a small and discrete habitat patch, suggests that simple reduction in the size of stands by natural senescence or land clearing/grazing would reduce genotypic diversity and could easily lead to the formation of monoclonal stands.

Implications for conservation of *A. loderi*

The highly fragmented distribution of *A. loderi* throughout most of its known range coupled with low levels of successful sexual recruitment within many stands means this species may have little capacity to respond to environmental change. In the short-term, asexual reproduction via suckering within especially the western stands, may continue to represent the strategy with which this species is able to best overcome the challenges imposed upon it by grazing or other impacts, such as reduced pollinator services. However, historically this reproductive tactic almost certainly formed a part of a bet-hedging strategy in which episodes of sexual recruitment could occur through outcrossing with neighbouring genets (Vallejo-Marín, Dorken, & Barrett, 2010). Currently, typically monoclonal western stands are isolated from other stands by > 10 km. Moreover, while the genotypically diverse eastern stands are probably the result of sexual reproduction and recruitment, it is unclear whether this is a continuing process.

Additional genetic surveys of adult stands and a range of experimental tests may provide novel insights and aid in the conservation of *A. loderi*. Most critically, surveys should be extended further northeast (to better estimate the scale of population subdivision within sexually generated stands), and west to further test the prediction that levels of clonality and polyploidy increase with increasing aridity, while providing further tests of the effect of the DR. Limited on-ground surveys and environmental data, such as the distribution of soils, indicate that the species probably extends north to White Cliffs (180 km from Kinchega NP) and west into South Australia (New South Wales Government, 2014; Atlas of

Living Australia, 2014). Ideally, a combination of chloroplast and nuclear DNA sequence data should be used to estimate the time of divergence of eastern, western, and southern lineages of this and other plant species (e.g. Byrne & Hankinson, 2012). Experimental tests are needed to characterise *A. loderi's* capacity for interbreeding among diploids and polyploids. To better understand geographic patterns of sexual reproduction and estimate mating system parameters, including outcrossing rates and rates of fertilization by pollen from other stands, further genetic surveys of adults and their seed are needed. Our results already indicate that any augmentation or replanting program must consider underlying patterns of allelic and life-history variation, and the possibility that individual stands are adapted to local conditions. We caution that, while eastern stands are a source of greater genetic diversity, eastern and western stands currently display strikingly different life-histories. Moreover, the restricted distribution of clones and variation in clonality and polyploidy suggests that smaller stands may be vulnerable and warrant individual management.

REFERENCES

Andrew RL, Miller JT, Peakall R. Crisp MD, Bayer RJ. 2003. Genetic, cytogenetic and morphological patterns in a mixed mulga population: Evidence for apomixis. *Australian Systematic Botany* **16:** 69-80.

Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA. 2007. Standardizing methods to address clonality in population studies. *Molecular Ecology* **16:** 5115-5139.

Arnaud-Haond S, Belkhir K. 2007. GENCLONE: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Resources* **7:** 15-17.

Atlas of Living Australia. 2014. *Acacia loderi* Maiden. http://bie.ala.org.au/species/Acacia+loderi

Auld TD. 1995. Soil seedbank patterns of four trees and shrubs from arid Australia. *Journal of Arid Environments*, **29**: 33-45.

Auld TD. 1993. The impact of grazing on regeneration of the shrub *Acacia carnei* in arid Australia. *Biological Conservation* **65**: 165-176.

Auld TD. 1990. Regeneration in populations of the arid zone plants *Acacia carnei* and *A. oswaldii*. *Proceedings of the Ecological Society of Australia* **16:** 267-272.

Auld TD, Denham AJ. 2001. Flora conservation issues at Kinchega National Park, western NSW. *Cunninghamia* **7:** 27-41.

Australian Government. 2014. Biodiversity, species profile and threats database. http://www.environment.gov.au/cgi-bin/sprat/public/sprat.pl

Barrett SCH, Kohn J. 1991. Genetics and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA & Holsinger KE, eds. Genetics and conservation of rare plants. Oxford University Press, New York, 3-30.

Beadle NCW. 1981. The Vegetation of Australia. Cambridge University Press, Melbourne.

Bowler JM. 1976. Aridity in Australia: age, origins and expression in aeolian landforms and sediments. *Earth-Science Reviews* **12:** 279-310.

Bowler JM, Kotsonis A, Lawrence CR. 2006. Environmental evolution of the Mallee region, western Murray Basin. *Proceedings of the Royal Society of Victoria* **118:** 161-210.

Byrne M. 1999. High genetic identities between three oil Mallee taxa, *Eucalyptus kochii* ssp. *kochii*, ssp. *plenissima* and *E. horistes*, based on nuclear RFLP analysis. *Heredity* **82:** 205-211.

Byrne M, Hopper SD. 2008. Granite outcrops as ancient islands in old landscapes: Evidence from the phylogeography and population genetics of *Eucalyptus caesia* (Myrtaceae) in Western Australia. *Biological Journal of the Linnean Society* **93:** 177-188.

Byrne M, Yeates DK, Joseph L, Kearney M, Bowler J, Williams MAJ, Cooper S, Donnellan SC, Keogh JS, Leys R, Melville J, Murphy DJ, Porch N, Wyrwoll K-

H. 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology* **17:** 4398-4417.

Byrne M, Hankinson M. 2012. Testing the variability of chloroplast sequences for plant phylogeography. *Australian Journal of Botany* **60:** 569-574.

Chen C, Durand E, Forbes F, François O. 2007. Bayesian clustering algorithms ascertaining spatial population structure: A new computer program and a comparison study. *Molecular Ecology Resources* **7:** 747-756.

Clarke LJ, Jardine DI, Byrne M, Shepherd K, Lowe AJ. 2012. Significant population genetic structure detected for a new and highly restricted species of *Atriplex* (Chenopodiaceae) from Western Australia, and implications for conservation management. *Australian Journal of Botany* **60:** 32-41.

Coates DJ. 2000. Defining conservation units in a rich and fragmented flora: Implications for the management of genetic resources and evolutionary processes in south-west Australian plants. *Australian Journal of Botany* **48:** 329-339.

Denham AJ, Auld TD. 2004. Survival and recruitment of seedlings and suckers of trees and shrubs of the Australian arid zone following habitat management and the outbreak of Rabbit Calicivirus Disease (RCD). *Austral Ecology* **29:** 585-599.

Dorken ME, Eckert CG. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology* **89:** 339-350.

Dunkerly DL. 2002. Infiltration rates and soil moisture in a groved mulga community near Alice Springs, arid central Australia: evidence for complex internal rainwater redistribution in a runoff–runon landscape. *Journal of Arid Environments* **51:** 199-219.

Durand E, Jay F, Gaggiotti OE, François O. 2009. Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology and Evolution* **26:** 1963-1973.

Eckert CG. 2001. The loss of sex in plants. *Evolutionary Ecology* **15:** 501-520.

Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* **24:** 217-242.

Evju M, Halvorsen R, Rydgren K, Austrheim G, Mysterud A. 2011. Effects of sheep grazing and temporal variability on population dynamics of the clonal herb *Geranium sylvaticum* in an alpine habitat. *Plant Ecology* **212:** 1299-1312.

Facelli JM, Brock DJ. 2000. Patch dynamics in arid lands: localized effects of *Acacia papyrocarpa* on soils and vegetation of open woodlands of South Australia. Ecography **23:** 479-491.

Falush D, Stephens M, Pritchard JK. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Resources* **7:** 574-578.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **164:** 1567-1587.

François O, Durand E. 2010. Spatially explicit Bayesian clustering models in population genetics. *Molecular Ecology Resources* **10:** 773-784.

Frankham, **R. 2005** Genetics and extinction. *Biological Conservation* **126:** 131-140.

Gale SJ. 1992. Long-term landscape evolution in Australia. *Earth Surface Processes and Landforms*, **17:** 323-343.

Ghazoul J. 2005. Pollen and seed dispersal among dispersed plants. *Biological Reviews of the Cambridge Philosophical Society* **80:** 413-443.

Goudet J. 1995. Fstat version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity* **86:** 485-486.

Greenville AC, Wardle GM, Dickman CR. 2012. Extreme climatic events drive mammal irruptions: Regression analysis of 100-year trends in desert rainfall and temperature. *Ecology and Evolution* **2:** 2645-2658.

Grice AC, Westoby M, Torpy C. 1994. Dynamics and population structure of *Acacia victoriae* Benth. *Australian Journal of Ecology* **19:** 10-16.

Hedrick PW. 2005. *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury.

Holman JE, Playford J. 2000. Molecular and morphological variation in the *Senna artemisioides* complex. *Australian Journal of Botany* **48:** 569-579.

Hopper SD. 1979. Biogeographic aspects of speciation in the southwest Australian flora. *Annual Review of Ecology and Systematics* **10:** 399-422.

Jensen JL, Bohunk AJ, Kelley ST. 2005. Isolation by distance, web service. *BMC Genetics* **6:** 6.

Kearney MR. 2003. Why is sex so unpopular in the Australian desert? *Trends in Ecology and Evolution* **18:** 605-607.

Kearney MR. 2005. Hybridization, glaciation and geographical parthenogenesis. *Trends in Ecology and Evolution* **20:** 495-502.

Kearney MR, Blacket MJ. 2008. The evolution of sexual and parthenogenetic *Warramaba*: A window onto Plio-Pleistocene diversification processes in an arid biome. *Molecular Ecology* **17:** 5257-5275.

Kennington WJ, James SH. 1997. Contrasting patterns of clonality in two closely related Mallee species from Western Australia, *Eucalyptus argutifolia* and *E. obtusiflora* (Myrtaceae). *Australian Journal of Botany* **45:** 679-689.

Kimoto C, Debano SJ, Thorp RW, Rao S, Stephen WP. 2012. Investigating temporal patterns of a native bee community in a remnant North American bunchgrass prairie using blue vane traps. *Journal of Insect Science* **12:** 108.

Loveless MD, Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* **15:** 65-95.

Maynard Smith J. 1978. *The Evolution of Sex*. Cambridge University Press, Cambridge.

Miller JT, Andrew RA, Maslin BR. 2002. Towards an understanding of variation in the Mulga complex (*Acacia aneura* and relatives). *Conservation Science Western Australia* **4:** 19-35.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* **70:** 3321-3323.

New South Wales Government. 2011. *Acacia loderi* Shrubland – endangered ecological community listing under the NSW threatened Species Conservation Act. http://www.environment.nsw.gov.au/determinations/AcaciaLoderiShrublandsEndComListing.htm

New South Wales Government. 2014. *Acacia loderi* Shrubland – profile. http://www.environment.nsw.gov.au/threatenedspeciesapp/profile.aspx?id=10017

O'Brien EK, Denham AJ, Ayre DJ. 2014. Patterns of genotypic diversity suggest a long history of clonality and population isolation in the Australian arid zone shrub *Acacia carneorum*. *Plant Ecology* **215:** 55-71.

Pepper M, Barquero MD, Whiting MJ, Keogh JS. 2014. A multi-locus molecular phylogeny for Australia's iconic Jacky Dragon (Agamidae: *Amphibolurus muricatus*): Phylogeographic structure along the Great Dividing Range of south-eastern Australia. *Molecular Phylogenetics and Evolution* **71:** 149-156.

Pickard J, Norris EH. 1994. The natural vegetation of north-western New South Wales: notes to accompany the 1:1 000 000 vegetation map sheet. *Cunninghamia* **3:** 423-464.

Pickard J. 1991. Sheep and rabbits - the biological chainsaws. *Search* **22:** 48-50.

Pillans B. 2007. Pre-Quaternary landscape inheritance in Australia. *Journal of Quaternary Science* **22:** 439-57.

Prider JN, Facelli JM. 2004. Interactive effects of drought and shade on three arid zone chenopod shrubs with contrasting distributions in relation to tree canopies. *Functional Ecology* **18:** 67-76.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945-959.

Roberts DG, Forrest CN, Denham AJ, Ayre DJ. 2013. Microsatellite markers for vulnerable Australian arid-zone *Acacias*. *Conservation Genetics Resources* **5:** 199-201.

Rossetto M, Jezierski G, Hopper SD, Dixon KW. 1999. Conservation genetics and clonality in two critically endangered eucalypts from the highly endemic south-western Australian flora. *Biological Conservation* **88:** 321-331.

Shafer ABA, Cullingham CI, Côté SD, Coltman DW. 2010. Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology* **19:** 4589-4621.

Smissen PJ, Melville J, Sumner J, Jessop TS. 2013. Mountain barriers and river conduits: Phylogeographical structure in a large, mobile lizard (Varanidae: *Varanus varius*) from eastern Australia. *Journal of Biogeography* **40:** 1729-1740.

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS. 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* **15:** 4261-4293.

Tiver F, Andrew MH. 1997. Relative effects of herbivory by sheep, rabbits, goats and kangaroos on recruitment and regeneration of shrubs and trees in eastern South Australia. *Journal of Applied Ecology* **34:** 903-14.

Tongway DJ, Ludwig JA. 1990. Vegetation and soil patterning in semi-arid mulga lands of eastern Australia. *Australian Journal of Ecology* **15:** 23-34.

Turchetto-Zolet AC, Pinheiro F, Salgueiro F, Palma-Silva C. 2013. Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology* **22:** 1193-1213.

Vallejo-Marín M, Dorken ME, Barrett SCH. 2010. The ecological and evolutionary consequences of clonality for plant mating. *Annual Review of Ecology, Evolution, and Systematics* **41:** 193-213.

Wardle GM, Pavey CR, Dickman CR. 2013. Greening of arid Australia: New insights from extreme years. *Austral Ecology* **38:** 731-740.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38:** 1358-1370.

Williams MAJ, De Deckker P, Adamson DA, Talbot MR. 1991. Episodic fluviatile, lacustrine and aeolian sedimentation in a late Quaternary desert margin system, central western New South Wales. In: Williams MAJ, De Deckker P, Kershaw AP eds. The Cainozoic in Australia: a re-appraisal of the evidence issue 18 of special publication. Geological Society of Australia, New South Wales, Sydney, Australia, 258-287.

Williams GC. 1975. *Sex and Evolution*. Princeton University Press, Princeton.

Wright S. 1969. Evolution and the genetics of populations, Volume 2: the theory of gene frequencies. University of Chicago Press, Chicago.

Yoshihara Y, Chimeddorj B, Buuveibaatar B, Lhagvasuren B, Takatsuki S. 2008. Effects of livestock grazing on pollination on a steppe in eastern Mongolia. *Biological Conservation* **141:** 2376-2386.

DATA ACCESSIBILITY

Spatial coordinates and microsatellite genotypes will be deposited in Dryad upon acceptance for publication.

AUTHOR CONTRIBUTIONS AND ACKNOWLEDGEMENTS

The authors are broadly interested in understanding the genetic consequences of life-history variation in plants. This contribution is part of a larger project that aims to evaluate causes of sexual reproductive failure in threatened Australian arid-zone acacias. Author contributions: D.J.A. and A.J.D. conceived the idea for the project and provided funding via a successful Australian Research Council Linkage grant (LP100100672); D.G.R., A.J.D and C.N.F. conducted field work; D.G.R. collected and analysed the genetic data; D.J.A and D.G.R drafted and wrote the manuscript; C.N.F and A.J.D. provided constructive feedback on all manuscript drafts. We thank New South Wales Office of Environment and Heritage, Department of Premier and Cabinet, for collection permits

TABLE CAPTIONS

Table 1. The number of *Acacia loderi* stems genotyped in each of 19 arid zone stands, *N*, number of unique multilocus genotypes (MLG) per stand, *G*, ratio (*G*-1)/(*N*-1), *R*, Simpsons complement, *D** and P_{sex}, probability of n (n = 1, 2, 3…i) replicates of a MLG deriving from distinct episodes of sexual reproduction.

Table 2. Mean (± SE) number of alleles, *A*, allelic richness, A_R, observed heterozygosity, H_o, and Nei's (1973) expected heterozygosity, H_e, and, the estimator, *f*, of the inbreeding coefficient, F_{IS} (Weir & Cockerham, 1984), with corresponding statistical significance for departure from Hardy-Weinberg equilibria, for 17 diploid arid-zone stands of *Acacia loderi*. Samples sizes, *N*, do not include replicates of multi-locus genotypes.

FIGURE CAPTIONS

Figure 1. Map of the study area, southeastern Australia (a). There were 9 Acacia loderi stands (labelled 1 – 9) east of the Darling River (DR) in western New South Wales and 10 stands west of the DR (10 – 19) (b). Refer to Table 1 for stand names. Satellite images (a) and (b) from Google Earth http://earth.google.com/

Figure 2. Ancestry estimates for Acacia loderi allowing visual representation of complex genetic population structure and separation of the sexual eastern and largely clonal western stands. Each bar represents an individual's genotype. The colour shading making up each bar represents the inferred proportion of membership of an individual's genotype to each of 10 possible genetic clusters. The vertical black lines bounds individuals collected within stands.

TABLES

Table 1. The number of *Acacia loderi* stems genotyped in each of 19 arid-zone stands, *N*, number of unique multilocus genotypes (MLG) per stand, *G*, ratio (*G*-1)/(*N*-1), *R*, Simpsons complement, *D** and P_{sex}, probability of n (n = 1, 2, 3…i) replicates of a MLG deriving from distinct episodes of sexual reproduction.

Stand	*N*	*G*	*R*	D^*	P_{sex},
East of the Darling River					
1. Emmdale	40	40	1	1	-
2. Manara	30	30	1	1	-
3. Ivanhoe-Menindee Road 1	28	26	0.926	0.995	< 0.001 [1] †
					< 0.001 [1]
4. Ivanhoe-Menindee Road 2	30	29	0.966	0.998	< 0.001 [1]
5. Ivanhoe-Menindee Road 3	30	30	1	1	-
6. Ivanhoe-Menindee Road 4	30	30	1	1	-
7. Pooncarie Loop Road 1	30	28	0.931	0.993	< 0.001 [2]
8. Pooncarie Loop Road 2	30	30	1	1	-
9. Mungo National Park	11	1	0	0	< 0.001 [10]
West of the Darling River					
10. Wilcannia	30	1	0	0	< 0.001 [29]
11. Broken Hill Road, Wirryilka	30	25	0.828	0.986	< 0.001 [1]
					< 0.001 [2]

					< 0.001 [1]
					< 0.001 [1]
12. Kinchega National Park, East-West Road	30[†]	-	-	-	-
13. Kinchega National Park, South Big Dune	8	2	0.143	0.536	< 0.001 [4]
					< 0.01 [2]
14. Kinchega National Park, Far North-West	51	1	0	0	< 0.001 [50]
15. Kinchega National Park, Omega	62	24	0.377	0.920	< 0.001 [2]
					< 0.001 [6]
					< 0.001 [1]
					< 0.001 [1]
					< 0.001 [8]
					< 0.001 [1]
					< 0.001 [3]
					< 0.001 [2]
					< 0.001 [1]
					< 0.001 [12]
					< 0.001 [1]
16. Kinchega National Park, Far South-West	29	1	0	0	< 0.001 [28]
17. Tandou	30	23	0.759	0.982	< 0.001 [1]
					< 0.001 [1]
					< 0.001 [1]

					< 0.001 [1]
					< 0.001 [1]
18. Middlecamp[†]	30	-	-	-	-
19. Popiltah	17	2	0.063	0.118	< 0.001 [15]

[†] Number of replicates of each MLG.

Table 2. Mean (± SE) number of alleles, A, allelic richness, A_R, observed heterozygosity, H_o, and Nei's (1973) expected heterozygosity, H_e, and, the estimator, f, of the inbreeding coefficient, F_{IS} (Weir & Cockerham, 1984), with corresponding statistical significance for departure from Hardy-Weinberg equilibria, for 17 diploid arid-zone stands of *Acacia loderi*. Samples sizes, N, do not include replicates of multi-locus genotypes.

Stand	N	A	A_R	H_o	H_e	F_{IS}	Prob. HWE
East of the Darling River							
Emmdale	40	10.0 (1.6)	4.5 (0.4)	0.547 (0.047)	0.780 (0.038)	0.313 (0.042)	< 0.001
Manara	30	8.8 (1.3)	4.4 (0.3)	0.488 (0.055)	0.755 (0.041)	0.369 (0.058)	< 0.001
Ivanhoe-Menindee Road 1	26	7.3 (1.0)	4.0 (0.3)	0.457 (0.051)	0.713 (0.036)	0.375 (0.066)	< 0.001
Ivanhoe-Menindee Road 2	29	8.5 (1.3)	4.2 (0.4)	0.457 (0.065)	0.721 (0.072)	0.351 (0.087)	< 0.001
Ivanhoe-Menindee Road 3	30	8.3 (1.1)	4.4 (0.4)	0.471 (0.030)	0.763 (0.047)	0.382 (0.054)	< 0.001
Ivanhoe-Menindee Road 4	30	8.3 (1.0)	4.3 (0.2)	0.533 (0.040)	0.750 (0.025)	0.292 (0.068)	< 0.001
Pooncarie Loop Road 1	28	6.5 (1.1)	3.7 (0.3)	0.540 (0.049)	0.696 (0.038)	0.237 (0.059)	< 0.001
Pooncarie Loop Road 2	30	8.8 (1.3)	4.2 (0.4)	0.471 (0.052)	0.708 (0.060)	0.332 (0.076)	< 0.001
Mungo National Park	4	1.4 (0.2)	1.4 (0.2)	0.375 (0.183)	0.188 (0.091)	-1.000 (0.000)	< 0.05
West of the Darling River							
Wilcannia	7	1.4 (0.2)	1.4 (0.2)	0.375 (0.183)	0.188 (0.091)	-1.000 (0.000)	< 0.001
Broken Hill Road, Wirryilka	25	8.1 (1.0)	4.0 (0.4)	0.435 (0.036)	0.690 (0.055)	0.358 (0.073)	< 0.001
Kinchega National Park, South Big Dune	4	1.6 (0.2)	1.6 (0.2)	0.500 (0.189)	0.313 (0.091)	-0.600 (0.400)	> 0.05
Kinchega National Park, Far North-West	5	1.6 (0.2)	1.6 (0.2)	0.625 (0.183)	0.313 (0.091)	-1.000 (0.000)	< 0.001
Kinchega National Park, Omega	25	5.8 (0.9)	3.4 (0.4)	0.422 (0.089)	0.584 (0.088)	0.296 (0.093)	< 0.001

Kinchega National Park, Far South-West	4	1.6 (0.2)	1.6 (0.2)	0.625 (0.183)	0.313 (0.091)	-1.000 (0.000)	< 0.001
Tandou	23	6.6 (0.7)	3.7 (0.3)	0.549 (0.049)	0.687 (0.039)	0.191 (0.105)	< 0.001
Popiltah	4	2.0 (0.3)	2.0 (0.3)	0.594 (0.156)	0.356 (0.088)	-0.533 (0.131)	< 0.001

FIGURES

Figure 1. Map of the study area, southeastern Australia (a). There were 9 Acacia loderi stands (labelled 1 – 9) east of the Darling River (DR) in western New South Wales and 10 stands west of the DR (10 – 19) (b). Refer to Table 1 for stand names. Satellite images (a) and (b) from Google Earth http://earth.google.com/

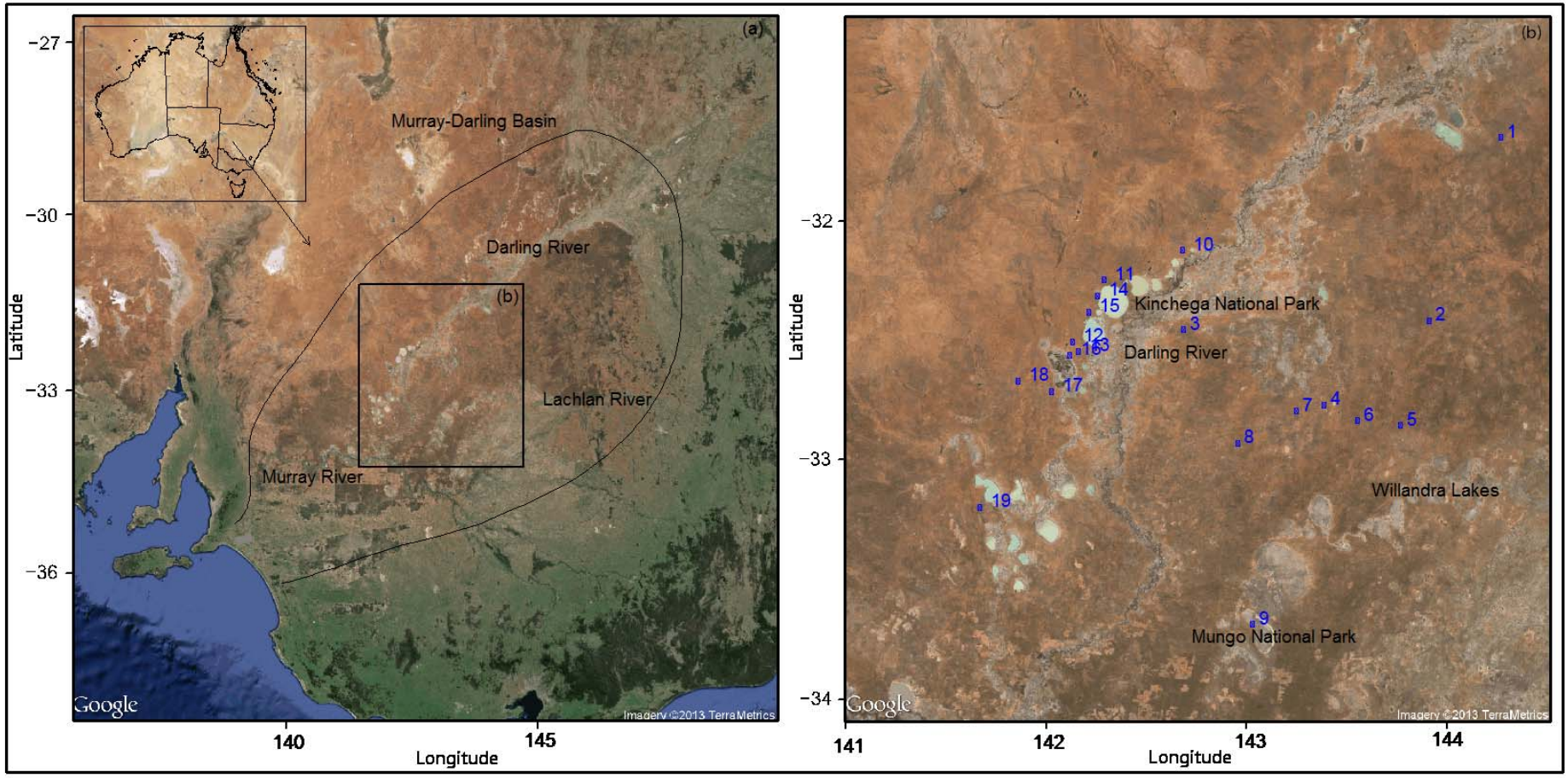
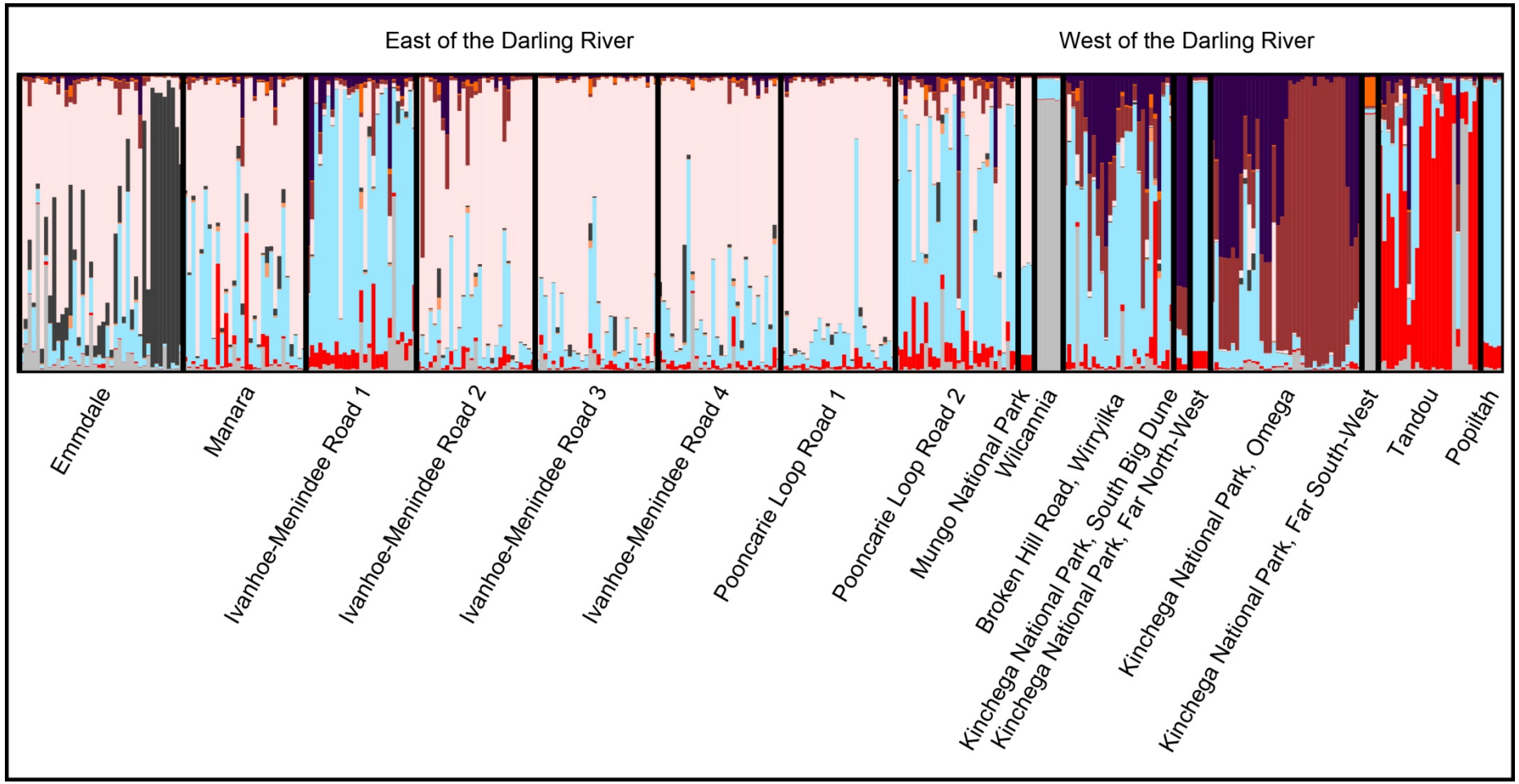


Figure 2. Ancestry estimates for Acacia loderi allowing visual representation of complex genetic population structure and separation of the sexual eastern and largely clonal western stands. Each bar represents an individual's genotype. The colour shading making up each bar represents the inferred proportion of membership of an individual's genotype to each of 10 possible genetic clusters. The vertical black lines bounds individuals collected within stands.



1